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# Composition of Montmorency Cherry Essence. 2. High-boiling Components

*SUMMARY—Volatile components of commercial Montmorency cherry essence boiling above ethanol were extracted from the essence by ether and concentrated by distillation. This concentrate was fractionated by gas chromatography. Individual components were identified using the methods of functional group analysis, gas co-chromatography, infrared analysis and mass spectrometry. The concentrations of the components in the original essence were estimated. The major components identified were n-propyl alcohol, isobutyl alcohol, isoamyl alcohol, and benzaldehyde. Minor components identified include n-butanol, n-hexanol, a hexenol, benzyl alcohol, α-terpineol, furfural, isoprene, myrcene and numerous higher terpenes, methyl benzoate, ethyl benzoate, benzyl acetate, ethyl caprylate, ethyl caprate, n-propyl benzoate, isobutyl benzoate, isoamyl benzoate and di-butyl phthalate.*

## INTRODUCTION

PREVIOUS WORKERS have identified several compounds with distinctive odors in cherries or cherry products. Mohler (1934) isolated an intermediate boiling fraction from cherry brandy that was considered to have the characteristic aroma of the brandy, as well as a high-boiling fraction that contained high boiling alcohols, benzaldehyde, coumarin and vanillin. Wasser et al. (1937) found terpineol in cherry wine. Nelson et al. (1939) isolated benzaldehyde from Montmorency cherry juice and also a yellow oil with a "lemon" aroma that they considered suggestive of geraniol.

In the first paper of this series Stinson et al. (1969) discussed the composition of the fraction of cherry essence boiling below ethanol. Various components found in this fraction had perceptible odors in the concentrations in which they were present in the original cherry essence. However, the fraction as a whole did not possess the distinctive aroma of cherry essence. This observation led to the belief that much of the distinguishing aroma would be found among the higher boiling components. This paper describes the isolation and identification of some of these higher boiling compounds.

## MATERIALS & METHODS

SEPARATION and identification of trace amounts of higher boiling compounds was complicated by the excessive amounts of methanol and ethanol present. This complication was overcome in the present investigation by solvent extraction with ether and concentration by distillation followed by preparative gas chromatography. Identification was chiefly by tandem gas chromatography and mass spectrometry supplemented by chemical methods of analysis and gas co-chromatography.

### Cherry essence

The cherry essence used in this study was the same as the material described previously (Stinson et al., 1969). It was a commercial grade 150-fold Montmorency cherry essence with a strong pleasant "cooked cherry" aroma. The product was shipped in 1 gal polyethylene bottles and stored in these containers at 34°C until used.

### Preparation of extract for qualitative gas chromatography

Sufficient reagent grade NaCl was added to 1500 ml portions of cherry essence to insure saturation. The mixture was extracted with 400 ml of freshly distilled reagent grade ether in a 2000-ml separatory funnel. The phases were separated and the extraction was repeated using two 300-ml portions of fresh ether. The aqueous layer, after removal of residual ether by distillation, had no perceptible cherry aroma.

The ether extracts were combined, dried with anhydrous $Na_2SO_4$ and distilled to 1/4 their volume using a 10 plate Oldershaw column. The distillation was continued with a 36 in. glass spiral Widmer column until the temperature at the head of the column reached 36°C. The residue at this point was largely ethanol with smaller amounts of the high-boiling components. Most of the remaining ether and much of the ethanol were removed by continued distillation using an 18 in. spinning band column (Nester/Faust Manufacturing Corp., Newark, Del.). The distillation was stopped when the temperature of the distillate reached 78°C.

The weight of the residue obtained in this manner from 11.7 kg of essence was 7.2 g, or 615 ppm of the original essence. This amount was smaller than the total of the quantities reported in Table 1 (approx. 1000 ppm) due to incomplete removal of low molecular weight alcohols during solvent extraction. The concentrations of the major alcohols listed in Table 1 were based upon analysis of the original essence.

### Gas chromatography

Analytical determinations were made with an F & M 720 gas-liquid chromatograph equipped with the F & M 700 module (F & M Corporation, Avondale, Pa.). This permitted the use of either thermal conductivity or flame ionization detectors. Analytical determinations were carried out primarily with the flame ionization detectors and the following columns:

(a) 10% Carbowax 20-M on 80–100 mesh Chromosorb Z, 6 ft × 1/8 in. O.D. (paired columns)
(b) 10% SE-30, ditto
(c) Carbowax 20-M, solid support not known, 50 ft × 1/8 in. O.D. (standard "Hi-Pak" column distributed by the F & M Corp.)

Preparative scale gas chromatographic separations using the thermal conductivity detectors were made to facilitate analysis with the mass spectrometer. The following column was used for this purpose: 20% SE-30 on 45–60 mesh Chromosorb W support, 8 ft × 1/4 in. O.D. The organic compounds emerging from the exit port of the gas chromatograph were divided into five fractions in the basis of order of emergence from the column. The material was condensed by passing the gas through U-tubes immersed in a dry ice-acetone bath. The gas chromatograph (an F & M 810) used in conjunction with the mass spectrometer was equipped with a flame ionization detector and a 1:100 stream splitter. The following columns were used:

(a) 20% SE-30 on 60–80 mesh Chromosorb Z, 8 ft × 1/4 in. O.D.
(b) 20% Carbowax 20-M, ditto

### Identification methods

The components of the fractions isolated by gas chromatography were identified by qualitative organic classification tests, mass spectrometric analysis, and gas co-chromatography.

Qualitative organic classification tests were used to indicate the presence of various functional groups. The mixtures were examined by gas chromatography before and after the reaction. Change in the relative peak heights indicated the presence of a particular functional group in the compound responsible for that peak.

The following classification tests described by Howard (1967) were used: carbonyl compounds, 1% hydroxylamine hydrochloride; unsaturated compounds and aldehydes, $KMnO_4$. The acetyl chloride test for alcohols was used to a certain extent but was not entirely satisfactory as extraneous peaks were introduced by the reagent and the ace-

tate derivatives that were formed.

The mass spectrometer used was model 21-103C manufactured by Consolidated Electrodynamics, Inc., Monrovia, California. This was used for the analysis of either pure samples isolated by gas chromatography or for the analysis of compounds present in the gas stream emerging from the gas chromatograph. These were conducted to the mass spectrometer inlet through heated conduit tubes. Identifications were made by matching the spectra of the unknown compounds with the reference spectra published by ASTM and API (Project 44). Only strong spectra showing positive identity were used. The mass spectra of known compounds obtained under similar conditions were compared with the spectra of the unknown compounds in several instances.

Additional information on identity was obtained by gas co-chromatography on both polar (Carbowax 20M) and nonpolar (SE-30) columns.

### Quantitative Determinations

The concentrations of the high-boiling components present in the Montmorency cherry essence were determined from their peak heights from direct gas chromatography of the commercial essence. Peak heights rather than peak areas were the parameters measured due to the small concentrations present. The components sufficiently abundant to be determined using the original essence included *n*-propyl alcohol, isobutyl alcohol, *n*-butyl alcohol, isoamyl alcohol, and benzaldehyde. *n*-Pentanol was used as an internal standard for determining the concentrations of these compounds.

Two solutions were prepared, one consisting of cherry essence and a known concentration of standard, and the other consisting of a synthetic mixture containing the compounds known to be present and the same standard. The ratios between the peak height of the compound and the internal standard were calculated for both the cherry essence and the synthetic mixture, and the concentrations were estimated by comparing the values of these two ratios.

Additional concentration was necessary to permit estimation of the concentrations of the minor components indicated in Table 1. A fresh concentrate was prepared under carefully controlled conditions from 100 ml of cherry essence using three successive ether extractions of 35, 25 and 25 ml of solvent. The extracts were combined, dried and concentrated to 1.5 ml by distillation. A standard solution was prepared containing known amounts of *n*-hexyl alcohol, furfural, *n*-heptyl alcohol, and isoamyl benzoate in ethanol.

*n*-Heptyl alcohol served as an internal standard for the determination of *n*-hexyl alcohol and furfural. Isoamyl benzoate was used as a reference peak for the estimation of the concentrations of the minor components of Fraction V. The concentration of isoamyl benzoate in Fraction V was estimated by comparing its peak height with the peak height of this compound in the standard solution described above. The concentrations of the remaining compounds of Fraction V given in Table 1 were estimated by comparing their peak heights with isoamyl benzoate. The assumption was made that the response of the system to equivalent amounts of the other compounds would be roughly proportional to the response to this compound.

Table 1—Neutral high-boiling components in Montmorency cherry essence.

| Fraction no. | Peak no. | Identity | Est. conc. in orig. essence, ppm | Method of identification[2] |
|---|---|---|---|---|
| 1 | 1 | acetaldehyde[2] | | MS |
| | 2 | acetone[2] | | MS |
| | 3 | ethyl acetate[2] | | MS |
| | 4 | ethanol[2] | | MS |
| | 5 | isoprene (tentative) | (Variable) | MS |
| | 6 | 1-propanol | 120[3] | MS, GC, CHEM |
| | 7 | 2-methyl-1-propanol (isobutyl alcohol) | 90[3] | MS, GC, CHEM |
| | 8 | 1-butanol | 6[3] | MS, GC, CHEM |
| 2 | 9 | 3-methyl-1-butanol (isoamyl alcohol) | 290[3] | MS, GC, CHEM |
| 3 | 14 | 1-hexanol | 1.7 | MS, GC |
| | 16 | a hexenol | Approx. 1 | MS |
| | 19 | furfural | 1.6 | MS, GC |
| 4 | 20 | benzaldehyde | 490[3] | MS, GC, CHEM |
| 5 | 23 | myrcene | 0.1 | MS, GC |
| | 25 | benzyl alcohol | Combined, | MS, GC |
| | 26 | methyl benzoate | Approx. 1.6 | MS, GC |
| | 27 | Mixt. of terpenes | 3.8 | MS |
| | 28 | ethyl benzoate + benzyl acetate + high mol. wt. material | 1.1 | MS, GC |
| | 30 | $\alpha$-terpineol + ethyl caprylate + unident. terpenes | 1.8 | MS, GC |
| | 32 | a benzyl alcohol derivative | 0.3 | MS |
| | 33 | *n*-propyl benzoate | 0.3 | MS, GC |
| | 35 | isobutyl benzoate | 0.2 | MS, GC |
| | 37 | ethyl decanoate | 0.3 | MS, GC |
| | 43 | isoamyl benzoate | 0.8 | MS, GC |
| | 45 | "BHT"-(butylated hydroxy-toluene) | 1.5 | MS |
| | 60 | di-butyl-phthalate | 0.40 | MS |

[1] MS = mass spectroscopy; GC = gas chromatography; CHEM = chemical derivatives.
[2] Low-boiling previously identified (see Stinson, et al., 1969).
[3] Concentrations obtained from original cherry essence.

## RESULTS & DISCUSSION

A GAS CHROMATOGRAPHIC analysis on the original essence was desired to avoid possible alteration in the ratios of the components present due to the extraction and concentration methods. Although most components were present in too small amounts to be detected, some compounds were sufficiently abundant to be measured without preliminary concentration. Figure 1 indicates the relative quantities of the most abundant materials in the Montmorency cherry essence emerging after ethanol. The conditions selected for this analysis achieved both adequate separation of the first components emerging after ethanol and volatilization of the higher-boiling compounds so that they emerged within a reasonable time.

The first two peaks observed in Figure 1 contained compounds earlier identified in cherry essence by Stinson, et al. (1969): Peak 1, acetaldehyde + other low boiling components; Peak 2, ethanol + methanol. The subsequent peaks were identified as Peak 3, *n*-propyl alcohol; Peak 4, isobutyl alcohol; Peak 5, isoamyl alcohol; and Peak 6, benzaldehyde. Due to the prominence of their peaks, the concentrations of these compounds could be determined from gas chromatography of the original essence.

A minor peak between isobutyl alcohol and isoamyl alcohol (not visible in this diagram) was *n*-butyl alcohol. The diminutive character of this peak as well as the other minor peaks between isoamyl alcohol and benzaldehyde required preliminary concentration (described in Materials and Methods) for their quantitative determination. The heights of the peaks emerging immediately after ethanol are slightly distorted due to trailing by ethanol. Subsequently, the quantitative analysis of these components were conducted at a lower temperature (110°) where separation was more complete and the distortion less significant.

The concentrate prepared for qualitative gas chromatography (7.9 ml of concentrate from 11.7 kg of essence) was separated by preparative gas chromatography into five fractions to facilitate isolation and identification of the components. The fractions were collected in the order in which they emerged from the SE-30 column upon programming at 2°/min between 70° and 230°C. Two very large peaks were collected as Fractions II

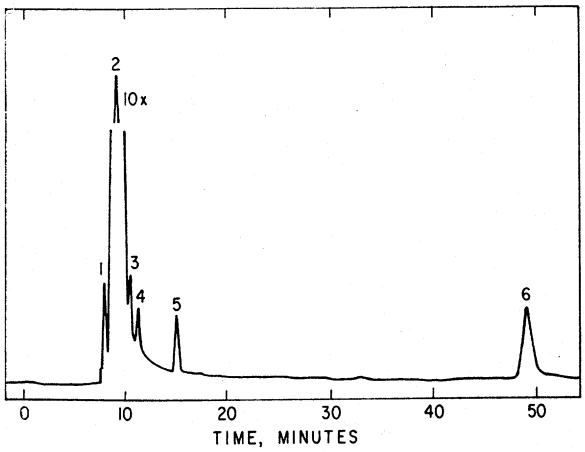


*Fig. 1—Chromatogram of original Montmorency cherry essence showing high-boiling components. 50 ft × 1/8 in. Carbowax 20 M column at 140°C.*

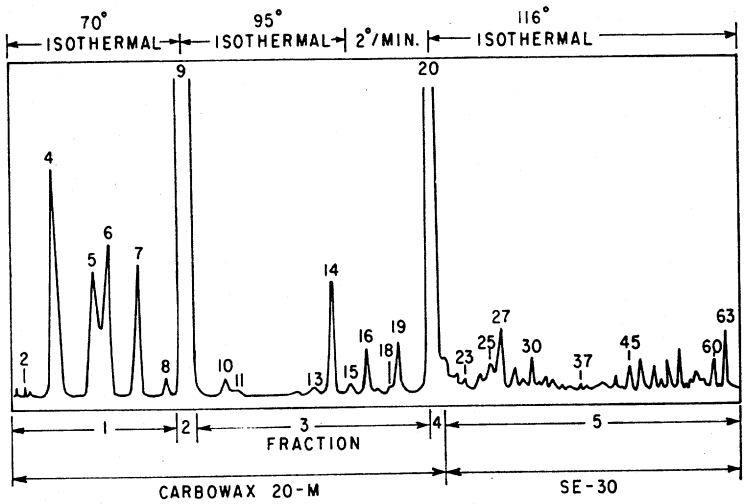


*Fig. 2—Composite chromatogram of fractions from concentrated extract of Montmorency cherry essence. 6 ft × 1/8 in. Carbowax 20 M column for Fractions I–IV; 6 ft × 1/8 in. SE-30 column for Fraction V. Temperatures as indicated.*

and IV, respectively. Fractions I and V consisted of those materials emerging before the first major peak and after the second major peak, respectively, while Fraction III consisted of the material emerging between the two major peaks. Fractions I to IV were colorless liquids. Fraction V was a yellow oil with an aroma resembling "lemon" or "cooked cherry."

Figure 2 is a composite showing the results of the analysis of these fractions on analytical columns. Columns containing Carbowax 20-M liquid phase were used for analysis of the first four fractions, while columns containing SE-30 were used for the analysis of the fifth fraction. The conditions used are given at the top of Figure 2.

The identification of the various peaks is indicated in Table 1. Trace amounts of acetaldehyde, acetone, ethyl acetate and ethanol were observed in Fraction I. These and other low-boiling compounds were reported earlier by Stinson et al. (1969). The main components of Fraction I were *n*-propyl alcohol, isobutyl alcohol, and isoamyl alcohol, together with a small quantity of *n*-butyl alcohol. Peak 5 gave a mass spectral pattern suggestive of methyl butadiene (isoprene). This peak was absent in the gas chromatography of the original cherry essence. This compound may be the result of thermal degradation of heat sensitive materials, possibly terpenes, during distillation and preparative gas chromatography. Brewster (1953) described the pyrolysis of a terpene to yield isoprene. No estimate was made of the concentration of the hydrocarbon in cherry essence.

Fraction II consisted almost entirely of isoamyl alcohol, but also contained trace amounts of the components of the preceding and following fractions.

Fraction III contained the components emerging between isoamyl alcohol and benzaldehyde. The compounds definitely identified in this region included *n*-hexyl alcohol and furfural. Another peak was tentatively identified on the basis of mass spectral analysis as a hexenol-1.

Fraction IV consisted almost entirely of benzaldehyde with trace amounts of the components of the preceding and following fractions. Gas chromatographic analysis of Fraction V revealed 42 peaks. Mass spectral analysis indicated that many, if not most, of these peaks contained more than one component.

Benzoic acid, not shown in the table, was identified in the benzaldehyde fraction (Fraction IV) where it may have been formed as the result of oxidation of the benzaldehyde. The presence of this compound in the original essence has not been confirmed. Two compounds, butylated hydroxytoluene (BHT) and di-butylphthalate, peaks 45 and 60, may be artifacts as these compounds are used in industry as an anti-oxidant and a plasticizer for plastics. Current investigations in our laboratory on untreated cherry juice is expected to establish whether these compounds are present in the original fruit.

It is not possible at this point to assign responsibility for the characteristic Montmorency cherry flavor to specific compounds. Many of the minor components of Fractions III and V were observed to have intense odors. Identification of these minor components is in progress. Work is also in progress on changes in composition during processing.

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